9. A. Z. Abyshev, P. P. Denisenko, N. P. Kostyuchenko, et al., Khim. Prir. Soedin., 45 (1972).
10. F. Bohlmann, M. Grenz, and C. Zdero, Chem. Ber., 108, 2955 (1975).
11. J. H. Totum and R. E. Berry, Phytochemistry, 18, 500 (1979).
12. G. K. Nikonov, Dokl. Akad. Nauk SSSR, 156, No. 5, 1210 (1964).
13. A. Z. Abyshev and P. P. Denisenko, Khim. Prir. Soedin., 114 (1972).
14. A. Z. Abyshev and I. V. Brodskii, Khim. Prir. Soedin., 574 (1974).
15. N. F. Gashimov, A. Z. Abyshev, A. A. Kagramanov, and L. I. Rozhkova, Khim. Prir. Soedin., 15 (1979).

# STRUCTURE AND STEREOCHEMISTRY OF THE COUMARINS OF *Ferula lehmannii*

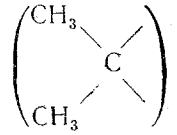
G. V. Sagitdinova, A. I. Saidkhodzhaev, and V. M. Malikov

UDC 547.9:582.89

Two new 7-hydroxycoumarins have been isolated from the roots of *Ferula lehmannii* (Lehmann's giant fennel): lehmferidin (I), $C_{24}H_{28}O_4$, $M^+$ 380, mp 173-174°C, and lehmferin (II), $C_{24}H_{30}O_4$, $M^+$ 382, mp 118-119°C; and badrakemin (III) has also been identified. The structures and configurations of the asymmetric centers of the new coumarins have been established on the basis of spectral characteristics and conversion into known substances.

From the roots of *Ferula lehmannii* Boiss. (Lehmann's giant fennel) collected in the Taldy-Kurgan province of KazSSR, by column chromatography we have isolated three crystalline substances which were assigned on the basis of their UV spectra to derivatives of 7-hydroxycoumarin: $C_{24}H_{28}O_4$, $M^+$ 380 (I); $C_{24}H_{30}O_4$, $M^+$ 382 (II); and $C_{24}H_{30}O_4$, $M^+$ 382 (III). On acid hydrolysis, all three substances formed umbelliferone (IV). According to their constants and spectral characteristics, substances (I) and (II) differed from known compounds, and we have called them lehmferidin (I) and lehmferin (II).

Substance (III) was identified from its physicochemical constants and IR spectrum as badrakemin [1].

The IR spectrum of substance (I) shows the following absorption bands ($cm^{-1}$): 3600-3620 (OH), 1730 (C=O of an α-pyrone), 2840-2980,  , 1680 (=), and 1560, 1520, and 1480 (aromatic nucleus).

In the PMR spectrum of (I) there are signals of three methyl groups (figures in ppm): 0.8 (6 H, s) and 1.0 (3 H, s); of a hemihydroxylic proton at 3.41 ppm (1 H, br.s, 1/2 Σ = 8.0 Hz); of the methylene protons of a $CH_2$—O—Cou grouping at 4.15 ppm (2 H, m); of an exomethylene group at 4.80 and 4.90 ppm (two singlets of 1 H each); of olefinic protons at 5.61 (1 H, d, J = 12 Hz) and 6.12 (1 H, d, J = 12 Hz). In the weak field appear the signals of the protons of the coumarin moiety of the molecule (ppm): 7.51 and 6.14 (doublets, 1 H each, J = 9.5 Hz) — $H_4$ and $H_3$; 6.80 (q, 1 H, $J_1$ = 9.5 and $J_2$ = 2.5 Hz); 7.34 (d, 1 H, J = 9.5 Hz) and 7.29 (1 H, J = 2.5 Hz) — doublets of $H_6$, $H_5$, and $H_8$, respectively.

The fragmentation of the ions in the mass spectrum of lehmferidin is typical for 7-hydroxycoumarins with an iresane substituent: 380 ($M^+$), 219 (M — O—Cou), 201 (M — O—Cou — $H_2O$)$^+$, and 162 (CouOH) [2].

When lehmferidin was acetylated with acetic anhydride in pyridine, a monoacetate (V) was obtained with the formula $C_{26}H_{30}O_5$ in the IR spectrum of which there was no absorption

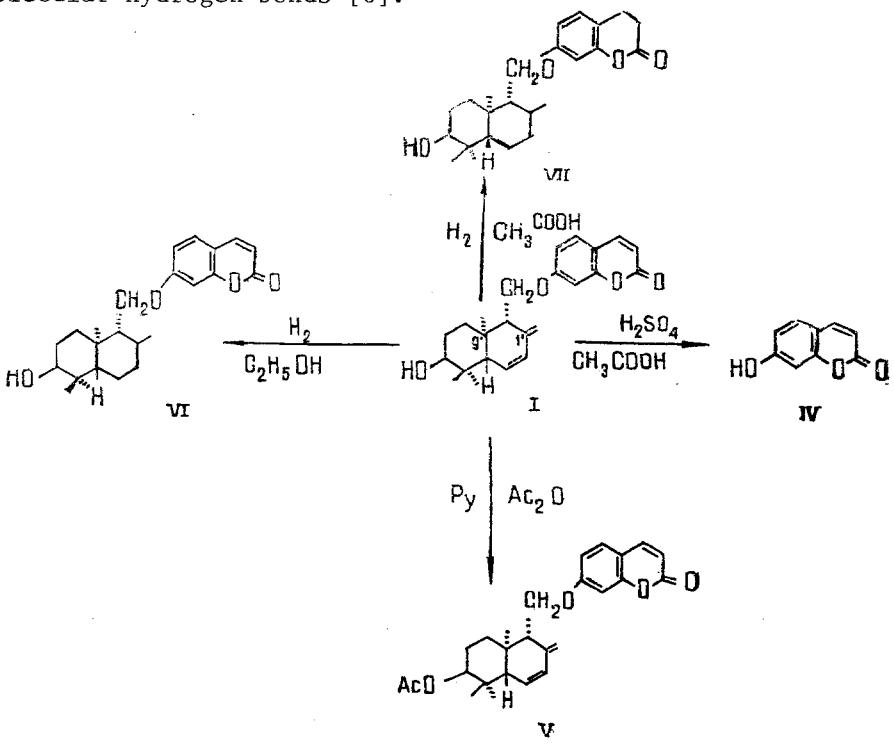
Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 709-712, November-December, 1983. Original article submitted December 13, 1982.

 0009-3130/83/1906-0672$07.50 

band of a hydroxy group, while in the PMR spectrum the signals of an acetyl group (1.98 ppm, s, 3 H) and of a gem-acyl proton (4.60 ppm, br.s, 1/2 Σ = 8 Hz) appeared. A comparison of the spectral characteristics and the chemical properties of lehmferin with those of the known 7-hydroxycoumarins with an iresane substituent showed lehmferidin was a new isomer of cauferidin, isolated previously from *Ferula conacaula* [3]. The substances differed only by the orientation of the hydroxy group, which in cauferidin is equatorial (3.27 ppm, q, $J_1$ = 9, $J_2$ = 6 Hz), and in lehmferidin is axial (3.41 ppm, br.s., 1/2Σ = 8 Hz). The olefinic proton in the PMR spectrum of (I) has an SSSC value (J = 12 Hz) showing that $C_{10}$–H has the axial orientation and the decalin nucleus the trans linkage.

In order to establish the stereochemistry of the asymmetric centers ($C_1$', $C_6$', $C_9$', and $C_{10}$') in the lehmferidin molecule we performed catalytic hydrogenation in the presence of platinum; and in ethanolic solution the reaction took place with the formation of a tetrahydro derivative (VI) $C_{24}H_{32}O_4$, while in acetic acid a hexahydro derivative $C_{24}H_{34}O_4$ (VII), identical with tetrahydroconferol [4, 5] according to its IR and PMR spectra and to a mixed melting point, was formed. Thus, lehmferidin has the structure and stereochemistry (I), i.e., the rings have trans-nonsteroid linkage, the orientation of $C_1$'–$CH_2OCou$ is equatorial, and the hydroxy group is axial, as in conferol [5].

The IR spectra of lehmferin (II) confirmed its assignment to the coumarin derivatives: 1510, 1565, 1615 $cm^{-1}$ (aromatic nucleus), 1695 $cm^{-1}$ (carbonyl of an α-pyrone), 3455 $cm^{-1}$ (hydroxy group). The shift of the carbonyl band to lower frequencies is a consequence of the formation of intramolecular hydrogen bonds [6].

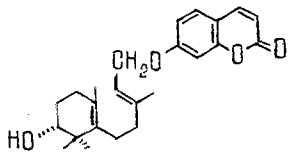



In the PMR spectrum of lehmferin signals are observed of two methyl groups at (ppm) 0.96 and 1.02 (s, 3 H each), of two methyls at a double bond – 1.55 and 1.73 ppm (s, 3 H each) – of an axial hemihydroxylic proton at 3.40 ppm (m, 1/2 Σ = 17 Hz, 1 H), of the methylene protons of a –$CH_2$–O–Cou– grouping at 4.51 ppm (d, J = 7.5 Hz, 2 H), and of an olefinic proton at 5.38 ppm (t, J = 7.5 Hz, 1 H). The identical SSCCs (J = 7.5 Hz) of the last-mentioned proton and of the –$CH_2$–O–Cou methylene protons permit the assumption that they are located on adjacent carbon atoms. In addition to the signals described, due to the terpenoid moiety of the molecule, the PMR spectrum of lehmferin contains signals due to the protons of 7-monosubstituted coumarin nucleus: doublets at (ppm): 7.52 and 6.13 (J = 10 Hz); a quartet at 6.70 ($J_1$ = 9, $J_2$ = 2 Hz), and doublets at 7.38 (J = 9.0 Hz), and 7.30 (J = 2 Hz).

With the composition $C_{24}H_{30}O_4$, lehmferin has two double bonds, one of them being trisubstituted since the PMR spectrum has the signals of two methyl groups at a double bond and at the same time only a one-proton signal of an olefinic proton. A comparison of the spectral characteristics of lehmferin with literature information shows that it is a new isomer (with respect to the position of the double bond) of kopetdaghin, isolated from *Ferula kopetdaghensis* [7].

The Adams hydrogenation of lehmferin led to the tetrahydro derivative (VIII), $C_{24}H_{34}O_4$, $M^+$ 386, identical with the product of the hydrogenation of farnesiferol B (according to IR and PMR spectra) obtained from an authentic sample.

Thus, lehmferin has the structure (II). It must be mentioned that such a substance has been obtained previously in an unresolvable mixture of two substances from the cyclization of umbelliprenin epoxide [8].



II

## EXPERIMENTAL

UV spectra were recorded on a Hitachi spectrophotometer (in ethanol), IR spectra on a UR-20 instrument (KBr), mass spectra on a MKh-1303, and PMR spectra on a JNM-4H-100/100 MHz spectrometer in $CDCl_3$ solution, 0 — HMDS.

The purity of the substances was checked and the course of the reactions was followed by TLC on Silufol (hexane—ethyl acetate (3:1) system).

The isolation and separation of the coumarins were carried out as described in [3].

Lehmferidin (I): $C_{24}H_{28}O_4$, mp 173-174°C, $M^+$ 380.

Lehmferin (II): $C_{24}H_{30}O_4$, mp 118-119°C, $M^+$ 382.

Badrakemin (III): $C_{24}H_{30}O_4$, mp 198-199°C, $M^+$ 382.

The acid hydrolysis of the coumarins was carried out by the usual method, by heating them in a mixture of acetic and sulfuric acids. In all three cases, umbelliferone $C_9H_6O_3$ (IV) was obtained with mp 230-232°C, $M^+$ 162.

Lehmferidin acetate (V), $C_{26}H_{30}O_5$, was obtained by acetylating lehmferidin with acetic anhydride in pyridine with heating. The reaction product was isolated in the usual way, $M^+$ 422. IR ($cm^{-1}$): 840, 895, 1030, 1130, 1250, 1350, 1380, 1520, 1615, 1710, 1730, 2950.

Tetrahydrolehmferidin (VI), $C_{24}H_{32}O_4$, mp 172-174°C, $M^+$ 384: 100 mg of lehmferidin was dissolved in 15 ml of ethanol and hydrogenated in the presence of 15 mg of platinum oxide for 1 h (Adams' method). After the end of the reaction, the catalyst was filtered off, the alcohol was distilled off, and the product was crystallized from ethyl acetate.

Hexahydrolehmferidin (VII), $C_{24}H_{34}O_4$, mp 199-200°C, $M^+$ 386: 100 mg of (I) was dissolved in 15 ml of acetic acid and hydrogenated in the presence of 20 mg of Adams platinum oxide for 1 h. The catalyst was filtered off, and the reaction mixture was diluted with water and treated with ether. The ethereal extract was washed with 5% sodium carbonate and with water, and it was dried with anhydrous magnesium sulfate and chromatographed on a column of silica gel. The eluant was hexane—ethyl acetate (5:1). This yielded 50 mg of (VII).

Tetrahydrolehmferin (VIII), $C_{24}H_{34}O_4$, $M^+$ 386, was obtained by hydrogenating lehmferin (I) in ethanol as described above.

IR ($cm^{-1}$): 840, 1010, 1235, 1380, 1460, 1510, 1555, 1615, 1725, 2855, 2930, 3200-3400.

Tetrahydrofarnesiferol B was obtained as described in [7].

## SUMMARY

Three 7-hydroxycoumarins have been isolated from the roots of *Ferula lehmannii*; one of them has been identified as badrakemin, and the structures and stereochemistries of the two new ones — lehmferidin and lehmferin — have been established by passage to known substances.

## LITERATURE CITED

1. N. P. Kir'yanov, Khim. Prir. Soedin., 363 (1967).
2. P. B. Terent'ev, P. I. Zakharov, G. K. Nikonov, T. Kh. Khasanov, and A. I. Saidkhodzhaev, Khim. Prir. Soedin., 207 (1977).

3. Z. A. Kuliev and T. Kh. Khasanov, Khim. Prir. Soedin., 327 (1978).
4. N. P. Kir'yalov and T. V. Bukreeva, Khim. Prir. Soedin., 425 (1973).
5. A. I. Saidkhodzhaev and V. M. Malikov, Khim. Prir. Soedin., 707 (1978).
6. M. E. Perel'son, Yu. N. Sheinker, and A. A. Savina, The Spectra and Structure of Coumarins, Chromones, and Xanthones [in Russian], Moscow (1975), p. 67.
7. Kh. M. Kamilov and G. K. Nikonov, Khim. Prir. Soedin., 442 (1972).
8. E. E. van Tamelen and R. M. Coates, Chem. Commun., 413 (1966).

# CHROMATOGRAPHIC SEPARATION OF ISOFLAVONES

I. I. Ozimina and A. B. Bandyukov

UDC 547.972.074:543.544

The optimum ratios of the components of the solvent systems have been determined with the aid of the simplex-grid method of experimental planning and the separation of a number of isoflavones has been carried out with the aid of these systems in thin layers of silica gel (Silufol plates); in addition, the chromatographic constants of the functional groups have been determined, which makes it possible to obtain the $\eta R_f$ values of compounds under investigation in an analysis of plant material by a mathematical method.

The detection of isoflavones in plant material is made difficult by the fact that many of them have no characteristic color reactions, and therefore chromatographic separation on paper or in a thin layer of adsorbent, elution of the spots from the chromatograms, and the recording of the absorption spectra from the eluates in the UV region is used for their investigation [1, 2].

To determine the absorption maxima it is necessary to be convinced that the separation of the substances on the chromatograms has taken place sharply. The observance of this condition is also necessary for the identification of the isoflavones in the presence of "markers."

Of the numerous group of flavonoids, the best-studied chromatographically are the flavones, flavonols, and flavanones. It has been established that the $R_f$ values of these compounds depend on the number of hydroxy groups and carbohydrate residues and their configuration [3, 4].

Usually the number of hydroxy groups is inversely proportional to the $R_f$ value; on methylation, $R_f$ falls, i.e., the following relationship exists:

$$R_f(x-\mathrm{OH}) < R_f(x-\mathrm{OCH_3}) < R_f(x-H) \quad [4].$$

We set ourselves the task of performing the optimization of the chromatographic separation of two-component mixtures of isoflavones in three-component systems of solvents in model experiments, using mathematical methods of experimental planning, and of determining the chromatographic constants of the functional groups of some isoflavones. Analysis of literature information on the chromatographic separation of isoflavones shows that in paper chromatography systems containing alcohols are used most frequently, and in thin-layer chromatography systems containing hydrophobic solvents: pentane, hexane, benzene, chloroform, and some others [2, 5-8].

On performing the chromatography of isoflavones in the alcoholic systems described in the literature (we always considered the separation of isoflavones in 17 systems), we established that in a number of cases the $R_f$ value is affected by the number of hydroxy groups but not by their distribution. The methoxylation or methylation of the isoflavone nucleus leads to a change in the $R_f$ value but, in a number of cases, it is impossible to distinguish compounds

Pyatigorsk Pharmaceutical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 712-716, November-December, 1983. Original article submitted November 23, 1982.

0009-3130/83/1906-0675$07.50  